Role of Endothelial-Mesenchymal Transition in the Development of Pulmonary Hypertension
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Abstract

**Rationale:** Endothelial-mesenchymal transition (EnMT) transition describes a process in which endothelial cells lose their endothelial cell phenotype/cell surface markers and gain mesenchymal or smooth muscle cell phenotype. This transition has been described in the developing heart and pulmonary artery. In addition to development, EnMT has been demonstrated in several pathologic processes. EnMT contributes to cardiac fibrosis and as a source of carcinoma-associated fibroblasts. Several in vitro studies have recently suggested that pulmonary endothelial cells can differentiate into myofibroblast and/or smooth muscle cells. While it has been postulated to occur in pulmonary vascular remodeling associated with pulmonary hypertension, in vivo evidence is lacking. **Methods:** Endothelial-mesenchymal transition will be characterized by genetically tagging endothelial cells (Tie2-cre-YFP) and following their fate after exposure to chronic hypoxia. **Results:** The endothelial specificity of the Tie2-cre-YFP mice was confirmed by immunohistochemistry. In addition, in normoxic conditions, flow cytometry demonstrated essentially complete overlap with a widely accepted endothelial marker, CD31 and no dual staining with alpha-smooth muscle cell actin (SMA) in room air controls. Interestingly, there was an increase in dual positive YFP and SMA after exposure to hypoxia. **Conclusions:** These results indicate that cells which were once endothelial in origin (Tie2-cre-YFP) have transitioned into mesenchymal cells (α-smooth muscle actin).

**Background**

- Genetic alterations in two members of the TGF β superfamily pathways, bone morphogenetic protein receptor II (BMPR II) and the TGF-β receptor I, ALK1, have been implicated in the pathogenesis of PH.
- Alteration in TGF β signaling attenuates the development of chronic hypoxic PH and MCT-PH
- TGF β signaling has been implicated in driving EnMT.

**Hypothesis**

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**TGFβ**

myofibroblasts

Schematic Development of PAH results from an imbalance in TGF signaling within endothelial cells. Specifically, enhanced TGF signaling promotes a phenotypic change that allows the cells to undergo endothelial-mesenchymal transition and contribute to the smooth muscle or myofibroblast population.

**Results**

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**Figure 1:** Abrogation of TGF β signaling attenuates chronic hypoxia-induced PH. A transgenic inducible (CMV-Cre) ROSA-loxSTOP-loxβGalβact mouse. Following induction with tamoxifen and exposure to chronic hypoxia, the CMV-Cre TGF β inducible mice developed less pulmonary hypertension (**p<0.001**). Tie2-cre transgenic mouse

**Figure 2:** A schematic of genetically tagged endothelial cells. Tie2-cre mice were tagged to ROSA-loxSTOP-loxβGalβact mice. In the resulting offspring, one mutated region allowed for the tagging of endothelial cells shown below. B. Lungs from the animals were inflated with 95% nitrogen and stained with anti-α-SMA (green), anti-smooth muscle actin (Dako, red), and DAPI. ROSA-loxSTOP-loxβGalβact

**Figure 3:** Flow Cytometry: Tie2-cre ROSA-βGalβact mice exposed to room air or chronic hypoxia. Lungs from the animals were stained with 0.9% agarose and stained with anti-GFP (coumarin, green), anti-α smooth muscle actin (Dako, red), and DAPI. Confocal microscopy (10X) was used to localize YFP and α-SMA expression. Within the small vessels (50-150μm) we observed dual staining only in the lungs from animals exposed to chronic hypoxia. (N=4)

**Figure 4:** Pulmonary artery smooth muscle cells (PASMCs) isolated from Tie2-cre-ROSA-YFP mice exposed to hypoxia or room air were cultured and stained for α-smooth muscle actin. Dual fluorescence was then demonstrated in the PASMCs isolated from animals exposed to chronic hypoxia whereas no YFP signal was detected from those obtained from room air controls. This suggests that α-SMA positive cells derived from cells of endothelial origin.

**Figure 5:** Immunofluorescent staining of small pulmonary vessels from Tie2-cre-ROSA-YFP mice exposed to room air or chronic hypoxia. Lungs from the animals were stained with 0.9% agarose and stained with anti-GFP (coumarin, green), anti-α smooth muscle actin (Dako, red), and DAPI. Confocal microscopy (10X) was used to localize YFP and α-SMA expression. Within the small vessels (50-150μm) we observed dual staining only in the lungs from animals exposed to chronic hypoxia. (N=4)

**Conclusions**

In these studies we have demonstrated that abrogation of TGF β signaling by inducibly knocking out TGF β receptor II attenuates the development of chronic hypoxia induced PH and hypothesize that increased TGF signaling is promoting endothelial to mesenchymal transition. Using the hypoxic model of pulmonary hypertension (CH-PH) with mice whose endothelial cells are genetically tagged, we have found: (1) a proportion of genetically labeled endothelial cells lose some of their endothelial cell characteristics. (2) some of the PASMCs cultured express YFP. (3) Co-localization of α-SMA and YFP by confocal microscopy in the remodeled pulmonary arteries suggesting that endothelial-mesenchymal transition is occurring in vivo. Experiments are currently being conducted to determine if these are endothelial cells or bone marrow derived.

**References**


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